

Ludger Sialic Acid Analysis Guide I:

NeuAc and NeuGc Quantitation

Sialic acid analysis is a regulatory requirement laid out in the ICH Q6B guidelines for characterisation of biopharmaceuticals. In particular, determination of relative levels of human and non-human type sialic acids (NeuAc/NANA and NeuGc/NGNA respectively) is important since some patients have high levels of anti-NeuGc antibodies which could lead to neutralization and rapid clearing of NeuGc-containing biopharmaceuticals (Nguyen et al, 2005). The significance of NeuAc and NeuGc when analysing glycoproteins means that it is often considered a critical quality attribute (CQA) for biotherapeutics.

A widely used method for determining the ratio of NeuAc to NeuGc is as follows:

- Release of sialic acid residues from the glycoprotein by mild acid hydrolysis.
- Fluorescent labeling of released sialic acids with 1,2-diamino-4,5-methylenoxybenzene (DMB).
- Relative quantitative analysis of DMB-labelled sialic acids by HPLC or UHPLC.

The LudgerTag™ DMB kit (Cat No. LT-KDMB-A1) provides all that is required to release sialic acids from glycoproteins and label them with DMB. The kit contains reagents and materials for up to 22 glycoprotein samples (50-100 µg of glycoprotein per sample). Each labeling should work with approximately 10 pmol up to 2.5 nmol sialic acids per sample.

Included in the kit are a sialic acid reference panel (containing Neu5Ac, Neu5Gc, Neu5,7Ac2, Neu5,Gc9Ac, Neu5, 9Ac2 and Neu 5,7, (8), 9Ac3Gc) and both NeuAc and NeuGc quantitative standards. These three standards can be labelled with DMB alongside the released sialic acid samples. Preparation of serial dilutions of the NeuAc and NeuGc quantitative standards enables quantitative analysis. Information on how to do this is explained in the LudgerTag™ DMB kit guide. The NeuAc and NeuGc quantitative standards are also available to purchase separately if required.

For positive controls, we recommend using fetuin glycoprotein which contains both NeuAc and NeuGc sialic acids. We also recommend a purified glycopeptide standard, the first in a range of Ludger BioQuant™ quantitative standards, as a positive control. This glycopeptide standard (Cat No. BQ-GPEP-A2G2S2-10U) is a complex biantennary N-linked glycan terminating in two N-acetylneuraminic acids. Using this standard will enable you to check the efficiency of glycan release, labeling and recovery and will give you confidence in the accuracy of your sialic acid measurements. Ludger also sells a standard which can be used for identification of Neu5,9Ac2. Water can be used as a negative control.

Reference:

Nguyen DH, Tangvoranuntakul P, and Varki A. (2005) Effects of Natural Human Antibodies against a Nonhuman Sialic Acid That Metabolically Incorporates into Activated and Malignant Immune Cells. *J Immunol* **175**:228-236.

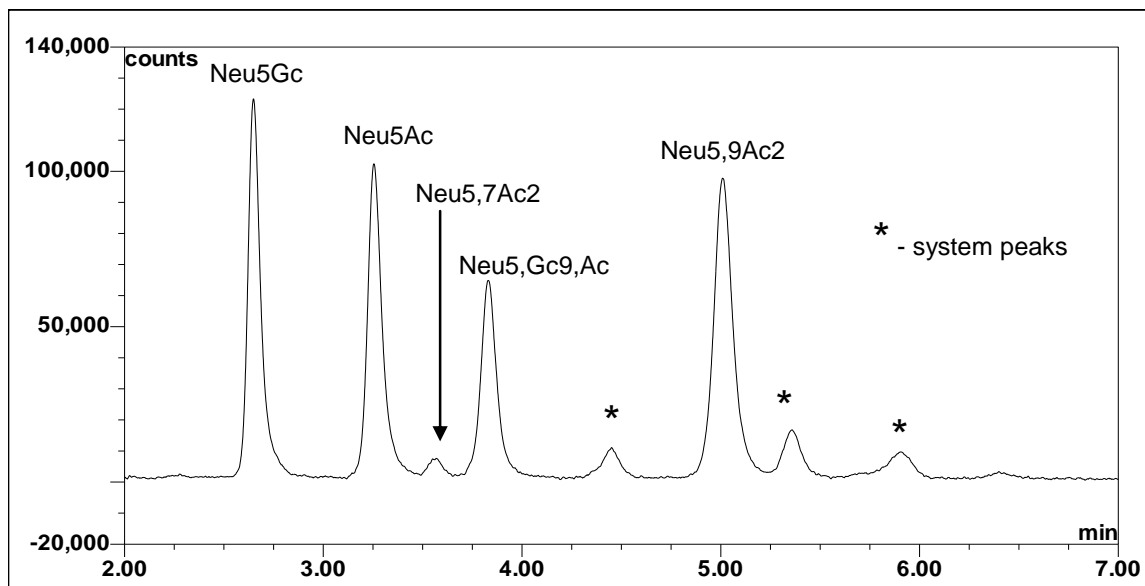


Figure 1: DMB-labeled sialic acid reference panel profiled on an LS-uR2 UHPLC column (Cat No. LS-UR2-2.1x100). The reference panel can be used as a system suitability standard prior to the sample run in order to check separation of glycans.

Ludger Products (these can be ordered via info@ludger.com)

Cat.No.

Release and label sialic acids with DMB:

LudgerTag™ DMB Sialic Acid Labelling Kit

LT-KDMB-A1

Sialylated Positive Controls:

Fetuin Glycoprotein (4 x 50 ug)

GCP-FET-50U-X4

Quantitative Glycopeptide Standard

BQ-GPEP-A2G2S2-10U

Neu5, 9 Ac2 standard

CM-NEU5,9,AC2-01

For HPLC analysis:

LudgerSep™ R1 HPLC Column

LS-R1-4.6x150

For UHPLC analysis:

LudgerSep™ uR2 UHPLC Column

LS-UR2-2.1x100